CXII. STUDIES ON THE PHYSIOLOGY OF PLAIN MUSCLE.

V. THE INFLUENCE OF CAFFEINE ON LACTIC ACID FORMATION.

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THE production of rigor in skeletal muscle by the action of caffeine, discovered by Cogswell [1852], has formed the subject of several investigations. Ransom [1911] showed that the rigor is accompanied by lactic acid production, and this was confirmed and the conditions of lactic acid formation further studied by Meyerhof [1921] and by Matsuoka [1924]. The alterations in the lactacidogen content of the muscle submitted to the action of caffeine was studied by Riesser and Neuschloss [1922], whilst the effects on heat production were studied by Hartree and Hill [1923]. From these researches it would seem that the effect of caffeine is greatly to accelerate the breakdown of glycogen and of lactacidogen into lactic acid, by unloosing a chain of processes similar to those which ensue upon stimulation of the muscle; in presence of oxygen, the oxygen usage of the muscle is greatly increased though there does not appear to be any restitution of lactacidogen.

The rigor which is produced in striated muscle by high concentrations of caffeine is irreversible, and is accompanied by destruction of the structure of the muscle fibres, which has been studied by Secher [1914].

In a recent publication [Evans, 1925], I hinted that caffeine did not appear to cause acceleration of lactic acid production in plain muscle, and it is the further investigation of this point which forms the subject of the present communication.

METHODS.

Plain muscle from the tortoise stomach was used; the stomachs of several animals were used for each experiment and the sampling and estimations were carried out as described in the former paper [Evans, 1925], except that the tissue was not frozen but was killed by ice-cold 4 % HCl saturated with NaCl.

The estimation of lactic acid by the modified Furth and Charnass method is not interfered with by the presence of caffeine, as the following experiments show.

Exp. 1.

Caffeine taken (mg.)	Lactic acid taken (mg.)	Lactic acid found (mg.)	Recovery %
15	2.45	2.40	98
15	2.45	2.35	96
30	2.45	2.18	89
0	2.45	2.2	90
0	2.45	2.25	92
0	2.45	2.2	90
0	2.70	2.60	96
0	2.70	2.62	97
0	2.70	2.39	89
100	0.0	0.0	—

In order to make quite certain that no effect of any kind was introduced by the presence of caffeine, in some of the experiments caffeine was added in equal amounts to all the samples, either before the experiment began, or after the tissue had been killed by acid. The same results were obtained when this was done as when caffeine was not added to the control samples.

RETARDATION OF LACTIC ACID FORMATION BY CAFFEINE.

When portions of plain muscle were suspended in (frog) Ringer's solution containing caffeine, and kept anaerobically (by $N/500$ NaCN or nitrogen), there was always a smaller production of lactic acid in a given time than in control samples in Ringer without caffeine under the same conditions. The inhibition of lactic acid formation varies in proportion to the caffeine concentration. In the following experiment, where there were very small amounts of caffeine, the reduction was not much beyond the amount of the experi-

Fig. 1.

mental error; but it is important to know that even with small concentrations of caffeine there is never any acceleration of lactic acid production, since some of Meyerhof's figures suggest that, even with striated muscle, the accelerated acid formation at lower concentrations may give place to a retarded rate when the concentration of caffeine is above a certain amount. This would probably be due to the fibres being killed quickly.

Exp. 2. Tissue samples about $3\frac{1}{2}$ g. in 5 cc. of solution at 13° .

Control tissue in Ringer + $N/500$ NaCN for 2 hours gave 0.057 % lactic acid (mean of two estimations).

Tissue in Ringer + 0.002 % caffeine + $N/500$ NaCN for 2 hours gave 0.052 % lactic acid.

Tissue in Ringer + 0.01 % caffeine + $N/500$ NaCN for 2 hours gave 0.054 % lactic acid.

When the amount of caffeine is greater, the inhibition is more pronounced and, as shown by Fig. 1, is roughly in linear relationship to the concentration

of caffeine present, which there varied from 0.05 to 0.4 %. In this experiment the samples of tissue (about 3 g.) were suspended in 5 cc. of the Ringer-cyanide solution for 2½ hours at 16°.

After more prolonged action the inhibition is often still more pronounced as the next experiment shows (see also Exp. 6).

Exp. 3. Samples about 3.5 g. in 10 cc. of Ringer's solution at 12° in atmosphere of nitrogen. Fresh "resting" control, 0.019 % lactic acid.

Period in solution	Lactic acid present in solution		Inhibition
	With no caffeine	With 0.3 % caffeine	
2.5 hours	0.036 %	0.029 %	20 %
5.0 "	0.058	0.039	33

It is evident, therefore, that the tissue is not killed at an early stage by the caffeine, thus causing the lactic acid production to stop short, and further proof of this will be seen later.

The retardation is seen equally well when the tissue samples are placed in phosphate solutions instead of in Ringer's solution, as was done in Exps. 4, 5 and 6.

Exp. 4. Samples suspended in M/15 Na₂HPO₄ solution at 15°, in air, without cyanide. Fresh control, 0.008 % lactic acid.

Duration	Lactic acid in		Retardation
	Phosphate alone	Phosphate + 0.4 % caffeine	
1 hour 25 minutes	0.047 %	0.034 %	27 %
4 hours	0.064	0.056	12
Exp. 5. Fresh control, 0.026 %.			
30 minutes	0.059	0.043	27
Exp. 6. (In nitrogen) fresh control, 0.027 %.			
1 hour	0.066	0.047	29
2 hours 20 minutes	0.100	0.067	33
4 hours	0.126	0.074	41

Fig. 2.

In order to exclude the possibility that the muscle of the tortoise is in any way peculiar in its behaviour towards caffeine, an experiment was carried out with plain and striated muscle of this animal with and without caffeine. Samples of about 5 g. of plain, and of about 2 g. of striated muscle were suspended in 10 cc. of $M/15$ Na_2HPO_4 solution, in air, at 17.5° , and the rates of lactic acid formation compared. The results are given in Fig. 2, and show that the striated muscle behaves just like that of the frog and higher animals, in exhibiting acceleration of lactic acid formation in presence of caffeine, whilst the plain muscle shows the reverse.

When placed in phosphate solutions of lower hydrogen ion concentrations, the retardation is still seen and to about the same extent, though in any case the rate of lactic acid formation is definitely greater in the more alkaline than in the more acid solutions, as I have shown previously [Evans, 1925].

Exp. 7. Samples in 5 cc. solution at 15° for 3 hours.

Resting control, 0.023 % lactic acid.

Phosphate solution	Lactic acid after 3 hours in	
	Solution without caffeine	Solution with 0.3 % caffeine
$M/15$ Na_2HPO_4 (p_{H} 9.0)	0.066 %	0.046 %
$M/15$ NaH_2PO_4 (p_{H} 4.5)	0.053	0.036
Retardation by caffeine at p_{H} 9.0 = 30 %.		
Retardation by caffeine at p_{H} 4.5 = 32 %.		

It is important to note that addition of caffeine to the solution in which the muscle is kept merely *retards* the formation of lactic acid and does not produce the effect by causing early death of the muscle before it has had time to generate the acid. This is further illustrated by the next experiment, which shows that if sufficient time is allowed to give a lactic acid maximum, then this is the same whether caffeine is present or not.

Exp. 8. Tissue kept in $M/15$ Na_2HPO_4 + $N/500$ NaCN .

Time	% lactic acid present in tissue kept in		
	Solution without caffeine	Solution with 0.3 % caffeine	Lactic acid % difference
0 (control)	0.023	—	—
3 hours	0.066	0.046	0.02
23 hours	0.122	0.119	0.003

Taken together, the experiments so far described indicate quite clearly that in presence of caffeine, the formation of lactic acid in plain muscle under anaerobic, or approximately anaerobic, conditions, is definitely slowed. What now will be the effect of caffeine on the oxidative recovery process in this tissue?

RELATION OF CAFFEINE TO THE RECOVERY PROCESS.

This question was attacked in the first instance by subjecting portions of tissue to nitrogen for some hours, with or without caffeine, and then studying the effect of removal to oxygen for longer periods, lactic acid estimations being made on samples at each stage. Some results are given in Exps. 9 and 10.

Exp. 9. Fresh control, 0.019 % lactic acid. Samples of the tissue suspended in Ringer's solutions, without and with 0.3 % of caffeine, were exposed to nitrogen at 12°. The tissue without caffeine, after 5 hours in N, contained 0.058 % lactic acid, whilst the sample with 0.3 % caffeine contained 0.039 % lactic acid. The tissues were then transferred to oxygen, with shaking (not very efficient) for 18 hours, after which the sample with no caffeine contained 0.052 %, and that with caffeine 0.045 % lactic acid.

There was thus a slight actual reduction of lactic acid in absence of caffeine, whilst in presence of caffeine there was a slight increase. One must remember, however, that this result might be compatible with the caffeine leaving the recovery process fundamentally unaffected, the slight difference being due to the greater velocity of the recovery process in absence of caffeine because of the higher initial concentration of lactic acid in that instance. If, therefore, the recovery processes were normal the difference in the final figures would be due to the natural acceleration of the process in the first instance.

In the next experiment an attempt was made to test this hypothesis.

Exp. 10. Samples of fresh tissue were exposed in the moist state to moist nitrogen, without addition of caffeine, for 3 hours at 15°. The initial content of lactic acid was 0.023 %, and that after 3 hours' exposure to nitrogen was 0.063 %. One sample was now transferred to 5 cc. of oxygenated Ringer, and kept in oxygen with occasional agitation for 20 hours. Another sample was similarly treated with oxygenated Ringer containing 0.3 % of caffeine, and exposed to oxygen also for 20 hours. The lactic acid contents were then found to be:

(a) Without caffeine = 0.082 %.

(b) With caffeine = 0.087 %.

In both cases there was therefore an increase in the lactic acid content, though not to such a high level as if oxygen had been withheld. The results of the experiment appear somewhat doubtful, but again suggest that the recovery process has not been greatly affected by the presence of caffeine.

The next experiment was made by comparing the lactic acid formation in presence of caffeine under anaerobic conditions with that under reasonably good conditions for oxygen access.

Exp. 11. Each sample (of about 4.5 g.) of tissue was suspended in 5 cc. Ringer's or phosphate solution containing 0.3 % of caffeine. One series was placed in bottles filled with oxygen, and was shaken briskly for 2½ hours at 14.5°. To the other series N/500 NaCN was added, and the sample left at rest also for 2½ hours at 14.5°. The results follow:

Tissue in	Lactic acid present in tissue treated with	
	Oxygen and caffeine	Cyanide and caffeine
Ringer (fresh control)	(0.024) %	(0.024) %
Ringer 2.5 hours	0.025	0.029
NaH ₂ PO ₄ 2.5 hours	0.025	0.041
Na ₂ HPO ₄ 2.5 hours	0.024	0.039

The experiment shows that when oxygenation is efficient, the lactic acid can be kept down to its initial value when caffeine is present, though in absence of oxygen respiration, there is, in spite of the presence of the caffeine, a definite increase. This experiment therefore also suggests that the recovery process is either accelerated slightly, which would not accord with the two previous experiments, or is unaltered by caffeine.

The clearest indication that the processes of oxidative recovery are unaltered by caffeine is obtained by observing the oxygen usages of the tissue

with and without caffeine, and comparing these with what happens with the tortoise striated muscle. The results of such an experiment are given in Fig. 3.

Fig. 3.

This figure shows the results of a microrespirometer experiment, the total oxygen usages being plotted against the time. The effect of caffeine on the tortoise striated muscle is precisely similar to that found on the frog's striated muscle by Meyerhof, namely, a large increase in the oxygen utilisation, particularly during the earlier periods, as the slope of the curve shows. On plain muscle, there appears to be no effect at all; at the end of 342 minutes the total oxygen used per g. at 15° was, in presence of caffeine 189 mm.³, and without caffeine 190.5 mm.³; at all intermediate times also, the amounts were as nearly identical as could be expected.

ACTION OF ARSENATE AND CAFFEINE.

I have shown in the previous publication that arsenates accelerate lactic acid formation in plain muscle, just as Meyerhof found them to do in striated muscle.

It was therefore of interest to know how caffeine and arsenate in conjunction would act on plain muscle.

Fig. 4.

Fig. 4 gives the result of an experiment in which portions of plain muscle were treated with Ringer's solution containing (a) 0.3 % sodium arsenate, (b) 0.3 % caffeine, (c) 0.3 % arsenate plus 0.3 % caffeine, (d) neither addition. The results shown in the figure prove that caffeine can exert a decidedly inhibitory effect on the arsenate.

DISCUSSION.

The difference in the action of caffeine on plain and on striated muscle is of some theoretical interest, particularly in view of the identical action of arsenates on the two tissues. If we assume that arsenate plays some rôle in accelerating the breakdown of hexosephosphate, as it does in fermentation and apparently in striated muscle, then it seems reasonable to suppose that it may play a similar part in plain muscle. But since caffeine has an opposite effect on the two varieties of muscle, it would appear that it does not act on the same link in the chain of reactions as arsenate does, but that the action is of a physico-chemical nature in the first instance and, in striated muscle, depends on its causing structural damage to the tissue and so starting the breakdown process further back than arsenate does. The investigations of Hoet and Marks [1926] have recently given grounds for the belief that the

onset of rigor (and perhaps of contraction too) in striated muscle depends upon the depletion of glycogen, and it would not be going far to postulate, as the underlying cause of contraction, the disturbance, in a reversible manner, of a state of physico-chemical equilibrium in which glycogen plays a part, the formation of lactic acid being a collateral and perhaps a synergic phenomenon. I have shown [Evans, 1926] that glycogen is the lactic acid precursor in plain as it is in striated muscle.

Secher found, in investigating the action of caffeine on the histological structure of muscle, that it had no effect on the unstriated muscles of the invertebrates. The destructive action therefore is absent. Yet caffeine *delays* lactic acid production, even that which is occasioned by the presence of arsenates. This suggests that the physical or chemical conditions of equilibrium in which glycogen is held may be different in the two forms of muscle, and in support of this view is the fact that even after depletion of its glycogen, plain muscle does not pass into rigor mortis. Disturbance of an equilibrium in which glycogen is concerned would thus perhaps appear to be less probable as an explanation of contraction in plain than in striated muscle, the evidence of the present paper so far being rather in favour of the lactic acid hypothesis in its direct form. Caffeine retards the spontaneous breakdown or liberation of the lactic acid precursor in plain muscle, and thus forestalls the possibility of formation of lactic acid, even when arsenate is there to accelerate reactions further on in the chain.

Finally, a few experiments which I have carried out show that caffeine causes relaxation of the tonus and cessation of movements of plain muscle, and also abolishes the contraction produced by such drugs as histamine, whilst arsenates cause contraction. It might be expected that other drugs which cause powerful contraction of plain muscle would also accelerate the lactic acid production, but up to the present I have not found this to be so with histamine or acetylcholine.

SUMMARY.

1. In presence of caffeine, in concentrations from 0.002 to 0.4 %, the rate of lactic acid formation in plain muscle under anaerobic conditions is diminished.
2. The retardation of lactic acid formation is present in phosphate solutions of p_H 4.5 and p_H 9.0. The effect is merely a delay, since ultimately the same lactic acid maximum is reached whether caffeine be present or not.
3. The oxidative recovery process is not affected by caffeine; nor is the oxygen usage of the fresh tissue altered.

4. In presence of caffeine, the effect of arsenates in accelerating lactic acid formation is reduced. The effect of caffeine is probably physical or physico-chemical.

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